SOCIAL GROUPINGS AND CIRCADIAN ACTIVITY OF THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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Until relatively recently little was known about the characteristics and controls of circadian rhythms of fishes (Schwassman, 1971a ; Thorpe, 1978). Investigations with a number of Arctic species have revealed that the circadian system of fishes is highly labile, adapting to the prevailing environmental conditions (Eriksson, 1978; Müller, 1978a). Studies from more temperate regions have shown that seasonal differences exist in the length of the free running circadian period of the locomotor activity of cyprinid fish (Kavaliers, 1978).

The majority of those studies have dealt with the circadian activity of single fish recorded primarily under constant darkness. However, in the wild many fishes are normally found in groups ranging from aggregates to shoals and schools. What effect group size has on the circadian activity and period of fish and other vertebrates is not well known (Aschoff, 1979). In a brief report Siegmund and Wolff (1973) did suggest that both intensity of illumination and group formation can affect the circadian activity of the European minnow, *Leucaspius delineatus.*

The Atlantic killifish, *Fundulus heteroclitus,* a species of fish that is widely used in physiological and behavioral research, shows evidence of endogenous circadian rhythms. Killifish display a circadian rhythm of color change under constant illumination (Kavaliers and Abbott, 1977). Davis and Bardach (1965) demonstrated a "time coordinated pre-feeding activity rhythm" in killifish that they suggested was determined by an endogenous circadian rhythm. Killifish also occur in shoals and aggregates and thus are an ideal species in which to examine the effects of social grouping on circadian rhythmicity. A shoal is considered to be a facultative social group of fish within which a variety of behavior takes place (Pitcher, 1979). The present study investigates the effects of group size as exemplified by single killifish, aggregates of five fish, and shoals of 25 fish on circadian activity and period length.

MATERIALS AND METHODS

Killifish were obtained from the Marine Biological Laboratory, Woods Hole, Mass., and placed in 120-liter opaque plastic tanks under a 12-hour light 12-hour dark cycle (LD 12 : 12) at 20° C (±2° C) and 20‰ salinity. At a random time of day fish were fed a commercial fish food (Purina Trout Chow, St. Louis, Mo.). Activity determinations were carried out in shielded, opaque, polyethylene tanks (125 × 165 × 145 cm) with a gravel substrate and a glass stand-pipe at a water depth of 90 cm. A constant water flow (15 ml/min) at 20° C (± 2° C) and 20‰ was provided by a regulated water supply. Illumination was provided by overhead fluorescent tubes (120 W Sylvania Gro-lite) and incandescent lights (45 W Sylvania), whose intensity could be controlled by neutral density filters (Kodak Wratten) and dimming controls.
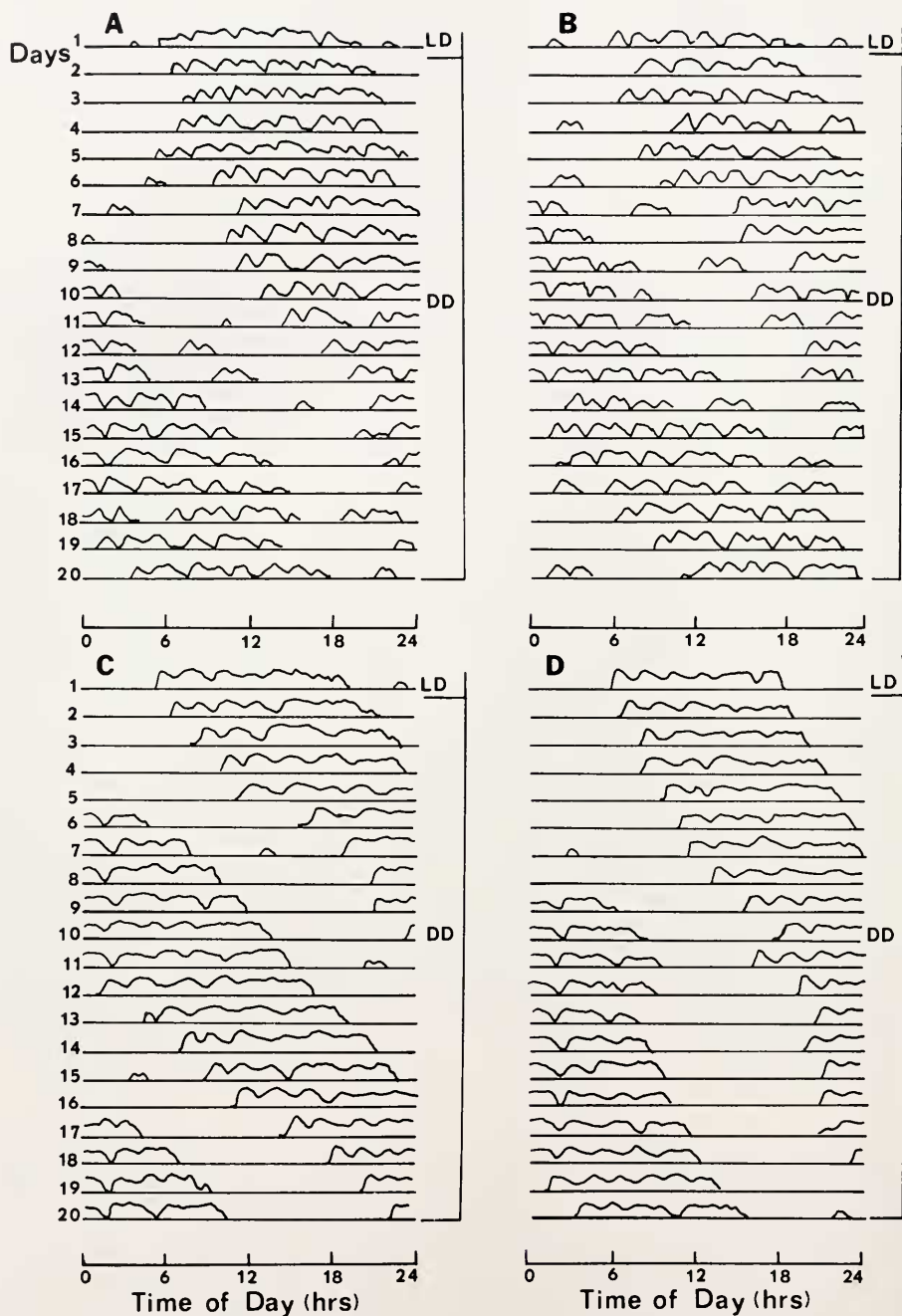
FIGURE 1. Examples of entrained (LD 12:12) and free running (DD, LL) activity totals of killifish. A. group of 5 fish under DD. B. group of 5 fish under LL at 100 lux. C. shoal of 25 fish under DD. D. shoal of 25 fish under LL at 100 lux. Successive days are from top to bottom. Hour 0 is at midnight Eastern Standard Time.

Total activity of individual and groups of fish was recorded by an externally-mounted ultrasonic system described in Kavaliers (1978). For computational purposes activity totals over 15 min were determined. Activity was recorded over 20 to 40 days for all of the individual and groups of fish examined. Visual inspection of the activity records permitted a subjective assessment of the presence or absence of circadian rhythmicity. Power spectral analysis was used to determine whether damped circadian rhythms occurred in the less precise records, as well as to obtain a more exact estimate of period length (Binkley, 1973; Kavaliers, 1978).

Experimental procedures

Ten killifish were individually placed under LD 12:12 for 10 to 15 days after which constant darkness (DD) was imposed and the length of the circadian period (τ) determined over 15 to 20 days. LD conditions were reimposed and circadian period determinations were repeated under constant illuminations (LL) of 1, 10 and 100 lux. Ten groups of 5 and 25 fish each were placed under LD 12:12 for 10 to 15 days after which DD and LL (1, 10, 100 lux) were imposed in a similar manner as for the individual fish. Circadian activity and period length were determined for each combination of group size and intensity of illumination.

RESULTS

Circadian activity of individual fish

Under LD conditions individual fish were light-active with maximal activity beginning 5 to 10 min before the onset of light and ending at the dark transition. With the imposition of DD and LL fish displayed a free-running circadian rhythm of activity that lost synchrony with the previous LD condition. Circadian activity of individual fish is described in detail elsewhere (Kavaliers, in prepartion). The relations between and dependencies of the circadian period length on the intensity of LL are summarized in Figure 3. Under DD there was a mean τ of 26.3 ± 0.20 hr . There were no significant differences in period values between different sexes or ages of fish.

Circadian activity of groups and shoals of fish

Five fish. When placed in groups of 5, the fish dispersed through the tank and behaved primarily as isolated individuals, occasionally forming a loose aggregation. Under LD the fish were light-active and under DD and LL they displayed free running circadian rhythms of activity (Figs. 1A, B). There was, however, a significantly more variable period ($P < 0.05$) than that of individual or groups of fish (± 0.50–0.75 hr for the aggregate as compared to ± 0.20–0.40 hr for the individuals and shoals). The increase in variability is shown by the broader and lower amplitude peak in the power spectra (Figs. 2A, B) and the greater variability in the free runs (Figs. 1A, B). Mean or individual group period length was not significantly influenced by LL ($P \leq 0.35$, t-test; 26.0 hr in DD and 25.7 hr in LL, Figs. 1A, B; 2A, B) or the intensity of illumination (Fig. 3).

Twenty-five fish. The group of 25 fish functioned as a facultative cohesive, nonpolarized, interacting social unit or shoal according to the definitions of Breder (1959) and Pitcher (1979). Fish were light active under LD and displayed free running
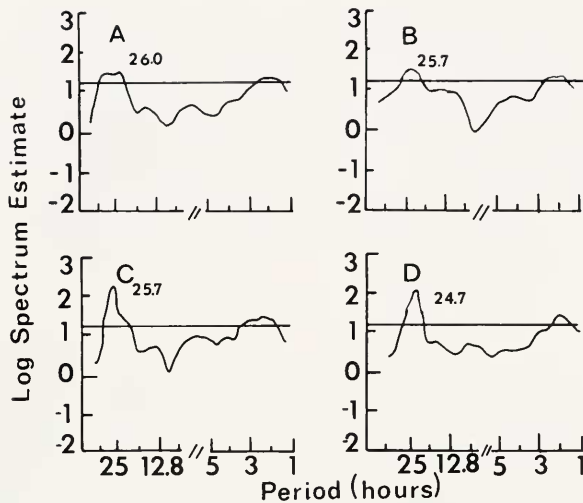
FIGURE 2. Power spectra derived from Fourier analysis of activity records in Figures 1A–D. The horizontal line indicates the upper 95% confidence limit. Only points (periods) above this level are significant. The circadian period associated with the peak is indicated beside.

circadian activity under DD as precise as that of single fish. τ values under DD (25.7 ± 0.20 hr Figs. 3C, 4C) were significantly different from those of single fish ($P < 0.01$, t-test). τ values of the shoals were dependent on the intensity of constant illumination (Fig. 3) (24.7 ± 0.20 hr for 100 lux LL Figs. 1C, D; 2C, D). The intensity or slope of the relationship between τ and LL was significantly different ($P < 0.01$, t-test) from that of the individual fish (Fig. 3). In addition the shoals of fish showed no change or increase in the variability of the period length with increasing intensity of illumination (Fig. 3). The τ values of shoals under LL were significantly less variable (F-test $P < 0.05$) than those of the individual fish.

DISCUSSION

The current study showed that the circadian activity and period length of killifish are affected by group size and social organization. Significant differences exist between the circadian activity and period of individual, aggregates, and shoals of fish, and circadian activity of killifish is affected by the intensity of illumination as per the circadian rule of Aschoff (1960).

According to the circadian rule, light-active animals show a shorter circadian period in LL than in DD, and in LL the period decreases with increasing intensity of illumination. However, in a subsequent reappraisal Aschoff (1979) indicated that various factors can strongly limit the validity of this generalization. In the present study the circadian period of single and shoals of fish complied to the circadian rule, while the group or aggregate of 5 fish failed to show any significant relations. However, the circadian periods of single and shoals of fish also differed significantly from one another. Maintenance of the fish under identical entraining conditions and determinations of τ at the same times of year minimized the possibility of annual variations in τ. In addition, the repeatability of τ values between LD treatments reduced the possibility of spontaneous variations in period length.

Previous determinations of the validity of the circadian rule for fishes have been
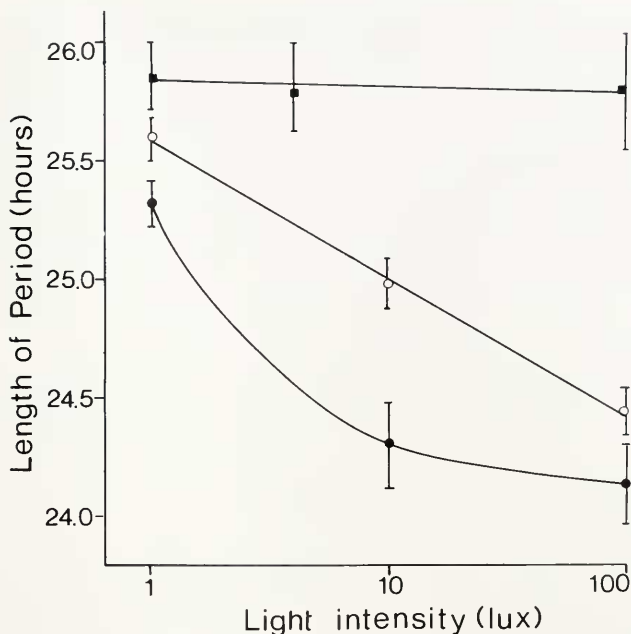
FIGURE 3. Effect of intensity of constant illumination (lux) on the free running circadian period length (τ) of single killifish (closed circles), groups of 5 fish (closed squares) and shoals of 25 fish (open circles). Each point is the mean of 10 indvduals or groups. The vertical line is the 95% confidence limit of the period estimates.

limited to a brief report on the circadian activity of *L. delineatus* (Siegmund and Wolff, 1973) and the frequency of electric discharge of Gymnotid electric fish (Schwassman, 1971b). The validity of the former determinations with *L. delineatus* can be questioned on both procedural and analytic grounds. The τ values were based on values obtained from 2 to 4 days, and were determined immediately after imposition of constant conditions. Sometimes the intial periods are transients (Pittendrigh and Daan, 1976). This may have been the case in the 1973 study of Siegmund and Wolff.

With single fish LL not only significantly increased τ values as compared to DD, but also increased the variability of the period. This is in contrast to the relatively more precise τ values reported for the locomotor activity of birds and mammals under LL. There is often an implicit assumption that the laboratory activity of fish is a single behavior comparable to the circadian parameters of birds and mammals. Fish laboratory activity, however, is a composite of behaviors normally occurring in the wild. Exploratory swimming, feeding, position adjustments and other appetitive and consummatory behaviors of killifish, each showing different reponses to light, cannot be considered as equivalent to the wheel running and perch hopping of mammals and birds, respectively. The latter two are artificial behaviors whose relations to either natural activity or other measures of activity are not established (Aschoff, 1979).

The type and extent of activity displayed by fish is also dependent on the number of fish or group size examined (Welty, 1934; Escobar, Minahan and Shaw, 1936; Symons, 1971). For many fishes and other vertebrates, examination of activity in the absence of conspecifics is an unusual, possibly stressful state.

Physiological and behavioral values determined from single fish need not necessarily bear close relations to values extrapolated from individuals in groups. In the present study the group of 5 fish dispersed through the tank, forming a loose aggregation. Their circadian activity was much more variable than that of single fish and had no significant relation to the intensity of illumination. This reponse of the group of 5 killifish differs from the results reported for *L. delineatus* (Siegmund and Wolff, 1973), though the latter may have formed a shoal. Dispersion of the killifish through the tank may have accentuated individual differences in activity giving a more variable period length. The differences in absolute period length from those of single fish and the lack of compliance to the circadian rule suggests the possibility of additional behavioral or physiological alterations. Aschoff (1979) has indicated that circadian period values are affected by experimental histories, by internal physiological state of the animal and, by external factors other than light, for example group size as shown in the present study.

It is generally assumed that forming a group or increasing the number of individuals in a group leads to a social facilitation of behavior (Clayton, 1978). The feeding behavior of fishes has provided evidence for group stimulation (Allee, 1934) or the so-called "group-effect" (Welty, 1934). This group facilitation arises from exogenous factors, being a function of tank size, density of fish and behavior being investigated (Allee, 1934; Schuett, 1934; Welty, 1934; Symons, 1971). Depending on the density of fish there may be either an increase or decrease in total and individual locomotor activity of groups of goldfish (Escobar, Minahan and Shaw, 1936). Social stimulation and facilitation of the entrainment of circadian activity is suggested for birds (Gwinner, 1966; Menaker and Eskin, 1966). With regard to free running activity a few data suggest that animals kept singly may have a longer period than animals kept in groups (Aschoff, 1979). These responses, however, are derived from experiments that did not involve either direct or visual contact between individuals or social interaction and the formation of groups.

In the present study the group of 25 fish formed a shoal. This was not directly an effect of tank size or density of fish, as in control experiments with larger tanks where density of fish was comparable to that of the individual or the group of 5, shoals were formed. There may be a minimum number of individuals necessary for endogenous social interactions and the formation of a shoal (Breder, 1959, 1976). The shoals of fish were diurnally activity with free running circadian activity under LL that was more precise than that of individual fish and dependent on the intensity of illumination. Müller (1978b) using photoelectric techniques reported that when single and schools of whitefish, *Coregonus laveretus*, were transferred to DD, the single fish became arhythmic while the shoals maintained rhythmicity for up to 10 days. This increase in precision may be considered as a form of social facilitation. However, the significant differences in period values between single and shoals of killifish suggest that the formation of a shoal results in a different behavioral entity with its own circadian characteristics. The circadian activity and period of a single fish cannot be considered as equivalent to, or representative of, an individual in an aggregate, group, or shoal of fish.

Circadian period values recorded after imposition of constant conditions are considered to be or represent history dependent "after-effects" of the previous entrainment conditions (Pittendrigh and Daan, 1976; Aschoff, 1979). Differences in τ values between single and shoals of fish may arise from socially and be-

haviorally induced differences in LD entrainment, in particular the facilitatory effects of shoal formation and break up at LD transitions.

Killifish as an estaurine species are exposed to temperature, salinity and tidal cycles as well as the daily light-dark cycle. What effects the former factors may have on modifying the circadian period and responses to light are not known. In view of the existence of seasonal changes in the circadian period of other fish (Kavaliers, 1978) the possibility of seasonal variations in the circadian period of single and groups of fish need also to be considered. The effects of social grouping and organization on the circadian rhythms of other fish and vertebrates needs to be further examined.

Summary

The effects of group size and intensity of constant illumination on the circadian locomotor activity of the Atlantic killifish, *Fundulus heteroclitus*, were examined. Individual fish displayed a free running circadian rhythm of locomotor activity under constant darkness (DD) and constant illumination (LL). The period length (τ) under DD was significantly greater than that under LL (26.3 ± 0.20 hr for DD as compared to 24.6 ± 0.40 hr at 100 lux). Period lengths were significantly dependent on the intensity of constant illumination. Groups of 5 fish displayed more variable circadian activity with no significant differences in τ values from DD and LL. The group of 25 fish formed a shoal and displayed precise circadian periods under DD and LL. The DD period was significantly greater than that under LL (25.7 ± 0.20 hr for DD as compared to 24.7 ± 0.20 hr for 100 lux). τ also decreased with increasing intensity of illumination. Circadian period lengths and relations to LL of shoaling fish were significantly different from those of individual fish. The circadian activity of a single fish cannot be considered as equivalent to, or representative of, an individual in a group or shoal of fish. Social groupings affect both the circadian activity and period length of killifish.

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